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Chronic Effects of Three Crude Oils on Oysters Suspended in Estuarine Ponds

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CHRONIC EFFECTS OF THREE CRUDE OILS ON OYSTERS SUSPENDED IN ESTUARINE PONDS

Carolyn Barszcz, Paul P. Yevich

Environmental Research Laboratory,
Narragansett, Rhode Island 02898

Lewis R. Brown

Department of Microbiology, Mississippi State University, Miss-
issippi State, Mississippi 39762

James D. Yarbrough, C. Douglas Minchew

Department of Zoology, Mississippi State University, Mississippi
State, Mississippi 39762

Gross and histological observations obtained from the study of oysters chronically exposed to single, low level (4 ppm) quantities of Empire Mix, Saudi Arabian, and Nigerian crude oils in estuarine ponds indicate a reduced intake and/or assimilation of food by the test animals. The tissues of many of the test animals, especially from the Saudi Arabian and Nigerian oil-treated ponds, were clear, watery, and emaciated in appearance, which was not the normal condition of oysters from the Gulf during the period of the samplings. Histologically, there were serious alterations in the connective tissues associated with the body wall, mantle, and food groove. The muscle and connective tissue fibers in these areas of many of the surviving oysters were swollen, translucent, homogeneous, structureless masses. These masses most nearly resembled the condition classically described as hyaline degeneration. In addition, there was a reduction in the development of the germinal epithelial tissues, indicating reduced reproductive potential.

INTRODUCTION

It is well documented that mollusks can survive short-term exposures to crude oils and can, in general, concentrate and depurate specific fractions of crude oils (Blumer et al., 1970; Lee et al., 1972; Stegeman and Teal, 1973). The chronic effects of crude oils and their degradation products on mollusks are far less clear. Much of the early literature either did not address the problem of chronic effects or concluded that, except for tainting of their flesh, there were few long-term effects which could be

Please send requests for reprints to Dr. C. Douglas Minchew.

attributed to petroleum hydrocarbons. However, more recent reports indicate that some hydrocarbons do cause alterations in the condition of certain mollusks. For example, Gilfillan (1973, 1975) reported an increase in respiration accompanied by a decrease in feeding and assimilation in 2 species of mussels, (*Mytilus edulis* and *Modiolus demissus*) exposed to 1 ppm crude oil.

Reduction in feeding was observed for *Mytilus edulis* exposed to low levels of the water accommodated fractions of no. 2 fuel oil (Gonzalez, 1976). No. 2 fuel oil has also been associated with reduced gonadal growth in *Mytilus edulis* (Blumer et al., 1971) and the development of gonadal tumors in *Mya arenaria* (Barry and Yevich, 1975). Mackin and Hopkins (1961) reported reduced growth and reduced glycogen content in oysters (*Crassostrea virginica*) exposed to bleedwater spray.

The present study attempts to address the problems associated with the chronic exposure of mollusk to crude oils by comparing the results obtained during the long-term (months) exposure of oyster to Empire Mix, Saudi Arabian, and Nigerian crude oils in estuarine pond ecosystems.

MATERIALS AND METHODS

Experimental Design

This study was conducted in a pilot-plant ecosystem consisting of 4 estuarine-ponds measuring 30.5 x 30.5 x 2.8 m which were constructed at the NASA National Space Technology Laboratory (NSTL) in Hancock County, MI. The ponds were filled with seawater (28 ppt) barged in from the Gulf of Mexico. The final salinities were adjusted to approximately 14 ppt with fresh well-water. Clumps of *Juncus roemerianus*, mixed occasionally with other species (e.g., *spartina*, *scirpus*, and *distichlis*), were dug from selected areas in the St. Louis Bay marsh (Hancock County, MS), transported to NSTL, and immediately planted along the banks of each of the ponds. The clumps included the natural muddy substrate along with its associated benthic fauna and microflora. These estuarine-pond ecosystems became established during the first year following their construction as evidenced by the development of a rich organic sediment, by the continual growth and regeneration of the marsh plants, and by the responsiveness of the plankton populations to salinity changes. During periods of high salinities (10-12 ppt), the planktonic populations were predominantly marine, while during periods of low salinities (5-6 ppt) they were predominantly freshwater and/or euryhaline. These shifts in the plankton populations were encouraging because they indicated that the planktonic populations in the ponds were responding to salinity changes in a manner similar to those in natural marshes.

Adjustments in the salinities of the ponds were occasionally required to counter salinity drops caused by rainfall. These adjustments were made by adding Na^+ , K^+ , Ca^{++} , Mg^{++} , Cl^- , and SO_4^{--} ions based on their ratios in Rila® Sea Salts Mix. The above ions were added in bulk quantities as salts (in the form of NaCl , CaCl_2 , MgSO_4 , MgCl_2 , and KCl). The

pond salinities averaged approximately 8 ppt for the duration of the study.

In June of 1975, oysters were collected from oyster beds near Ocean Springs, MS, and placed in suspended cages in the corners of each of the 4 ponds (100/corner; 400/pond). The cages were placed on supports on the sloping sides of the ponds so that the suspended oysters were approximately 30 cm beneath the surface of the water and approximately 30 cm (shallow side) to 60 cm (deep side) above the pond bottom. The oysters were suspended in baskets to avoid possible mortalities associated with silting, to assure easy access for routine monitoring, and to restrict their exposure to those crude oil components which could be obtained from the water column (dissolved, suspended, or attached to particulate matter, and so on).

In July, 1975 three of the ponds were treated with either Empire Mix, Saudi Arabian, or Nigerian crude oil at a level of 22.6 l/pond, while the fourth pond was retained as a control. The oil was spilled by sinking it in the middle of the respective ponds in weighted, 1 gallon plastic jugs which were slit just prior to their release. The oil bubbled slowly to the surface, producing a sheen which was visible for several days. The winds at first moved the slicks toward the southeast corners of the ponds. After several days, however, oil was visible in the sediments and on the grasses along all sides of the ponds, although its distribution did not appear even. The amount of oil released in each pond represented a very small spill. If this amount of oil had been evenly distributed onto the surface of the pond, an oil slick of 0.01-0.02 mm in thickness would have resulted. On the other hand, if all of the oil added to each pond had been equally distributed throughout the water column, a concentration of 4.0 mg/l would have been obtained.

Sediment, water, and oyster samples were taken at irregular intervals following the treatment of the ponds with crude oil. These samples were analyzed in methanol and water using a liquid chromatograph equipped with a UV detector set at 277 nm. In all cases, the values were corrected for the presence of biogenic hydrocarbons by subtracting control values.

Water samples were taken daily for the first 6 days following the oil spill and again after 12, 15, and 21 days. Samples were collected in 1 l glass bottles, placed in ice, and transported back to the laboratory for analysis.

Sediment samples were taken approximately 1, 2, and 8 weeks after the oil spills and again after 9 months. The samples were placed in glass jars and frozen for later analysis.

Oyster samples were taken approximately 2, 7, 8, 11, and 12 weeks following the oil spills and again after 9 months. They were transported live back to the laboratory, where they were subsequently sacrificed and analyzed.

This experiment was originally designed to compare the chronic toxicity of the 3 oils to the oysters and to determine whether a relationship existed between the oysters' resident microflora and their exposure to the respective crude oils. This design called for the removal of 40 oysters (10/corner) from each pond at each sampling period. Observations made

during the third sampling period (3 months following the oil spills) indicated that the oysters in the Saudi Arabian- and Nigerian-treated ponds were in poorer condition than those in the Empire Mix-treated and control ponds. Based on these observations and on the failure to obtain meaningful results from the microbial studies, the objectives of the study were reoriented, and the microbial samplings were suspended. A total of 180 of the original 400 oysters had been removed by this time. Those remaining were left undisturbed until the experiment was terminated in April, 1976.

Histopathological Materials and Methods

Live oysters chosen for histological analysis were removed from the ponds, shucked, and placed immediately into jars containing Helly's fixative. After 10-15 minutes, they were removed, sectioned as needed to allow proper fixation, and returned to the fixative for 24 hr. They were then removed from the fixative, placed in tissue capsules, and washed for 24 hr. in running tapwater. The tissues were then processed in an Auto-technicon using Technicon S-29 dehydrant and S-29 clearing agent, and finally embedded in Paraplast. The tissue blocks were sectioned at 6 microns and stained with Harris' hematoxylin and eosin for examination by light microscope. A portion of the slides and tissue blocks was sent to the Environmental Research Laboratory at Narragansett, RI for confirmation of the observed results. To help determine the nature of the changes observed in the organs and tissues, some blocks were re-cut and slides stained with Heidenhains' Azocarmine-aniline blue, Fraser-Lendrum's fibrin stain, Mallory's phosphotungstic acid hematoxylin, and/or Movat's pentachrome stain.

RESULTS

Chemical Analysis

The analysis of water samples following treatment of the ponds with crude oil failed to produce meaningful data. The level of oil in the ponds was below the level which could be separated from biogenic hydrocarbons using the methods available at that time. However, the results of numerous laboratory experiments using the same oils indicated that the water columns of the ponds probably contained less than 1 mg/l of oil, with the highest concentrations associated with the water directly under the oil slicks.

Analysis of pond sediments indicated that the oil was distributed in them in a checkerboard fashion, with some areas containing no measurable petroleum hydrocarbons and others containing as much as 32 $\mu\text{g/g}$. Again, as with water samples, interpretation of the results was made difficult by interference with biogenic hydrocarbons. On the average, the amount of oil contained in the sediments was estimated to be less than 1 $\mu\text{g/g}$. Oil was still detectable in the sediments of all 3 ponds after 9 months.

TABLE 1. Average Concentration of Petroleum Hydrocarbons Present in Oysters Chronically Exposed to 3 Crude Oils^a

	Pond ^c	# oysters/sample	µg oil/g tissue ^d
23 July 75 ^b	2	8	0
	3	8	0
	4	8	0
	5	8	0
7 Aug. 75	2	40	0
	3	40	200
	4	40	68
	5	40	55
8 Sept. 75	2	40	0
	3	40	18
	4	40	4
	5	40	10
16 Sept. 75	2	40	0
	3	40	10
	4	40	9
	5	40	20
1 Oct. 75	2	40	0
	3	40	24
	4	40	14
	5	40	15
11 Oct. 75	2	5	0
	3	5	20
	4	5	10
	5	5	9
19 April 76	2	32	0
	3	34	8
	4	20	2
	5	25	2

^aDeterminations made using a liquid chromatograph equipped with UV detector set at 277 NM.

^bPre-spill.

^cPond 2 = control; 3 = Saudi Arabian treated; 4 = Nigerian treated; 5 = Empire Mix treated.

^dThe average concentration of aromatic hydrocarbons present in control oysters (biogenic) was subtracted from the value obtained for treated oysters to get an estimation of the petroleum hydrocarbons in the samples.

The results given in Table 1 indicate that the oysters suspended in baskets in the water columns of the ponds were able to concentrate significant amounts of petroleum hydrocarbons. The majority of the oysters analyzed during the first 3 months contained oil. However, the levels of oil in the oysters removed after 9 months was variable. Some contained no measurable hydrocarbons, while others contained levels up to 8 $\mu\text{g/g}$.

Mortalities

If the total number of oysters stocked (400/pond) is considered, the respective mortalities were 23 percent in the control pond, 54 percent in the Saudi Arabian-treated pond, 50 percent in the Nigerian-treated pond, and 31 percent in the Empire Mix-treated pond. If, on the other hand, those oysters removed for microbial analysis are not considered (180/pond), the mortalities were 42 percent in the control pond, 85 percent in the Saudi Arabian-treated pond, 91 percent in the Nigerian-treated pond and 58 percent in the Empire Mix-treated pond. In either case, the percent mortalities for oysters in the Saudi Arabian- and Nigerian-treated ponds were higher than those in the Empire Mix-treated and control ponds.

Gross Examination

The following gross observations were obtained upon examination of the oysters following the termination of the experiment. The oysters from the control ponds had 2-5 mm new shell growth, were creamy white in color, and generally appeared fat (Fig 1). The oysters from the Empire Mix-treated pond had some new shell growth (usually < 2 mm), had less tissue color than the controls, and were slightly watery in appearance. The surviving oysters in the Saudi Arabian- and Nigerian-treated ponds were in extremely poor condition. They had no new shell growth, were gray in color, and were translucent, watery, and emaciated, in appearance (Fig. 2).

Microscopic Examination

The histopathological examination of a number of the surviving oysters from each pond substantiated the gross pathological findings. That is, the control oysters were in the best condition, followed by the Empire Mix-treated oysters, the Saudi Arabian-treated oysters, and finally by the Nigerian-treated oysters, which were uniformly in poorer condition than all other groups (Table 2). Differences between the oysters were noted primarily in the condition of the reproductive tracts, Leydig cells, and digestive diverticula (DD), and in the number and variety of parasites. While there was a variation in the condition of the oysters from any one pond, the differences in the condition of the oysters from different ponds was even greater. The following specific histopathological observations were made.



FIGURE 1. Photograph of a typical oyster removed from the control pond after 9 months (Empire Mix treated oysters were similar in appearance).

Control oysters. About one-half of the 15 oysters from the control pond that were examined microscopically had some development of the reproductive tract. In some of the animals there were 2 to 4 layers of germinal epithelium with some mature sperm in the center of the follicles and ducts, while others had ova in various stages of development along the follicle walls. The rest of the oysters in this group had only 1 layer of germinal epithelium, but it extended the full length of the follicle or duct wall and appeared normal (Fig. 3).

The Leydig or connective tissue cells were mostly in good condition throughout the body mass; that is, normal cell walls were present, and



FIGURE 2. Photograph of a typical oyster removed from the Saudi Arabian treated pond after 9 months (Nigerian treated oysters were similar in appearance).

many had the remnants of a pink-staining cytoplasm. In some animals the Leydig cells of the labial palps and along the periphery of the body were disrupted, with a loss of cell walls and of the pink cytoplasm. However, the disruption was considered within normal limitations for this stage of the reproductive cycle.

None of the digestive cells of the tubules of the digestive diverticula of these oysters were completely full of granules. While some of the digestive cells tended toward columnar, most were cuboidal, with a few tubules having almost squamous epithelium. Most of the ducts had a normal epithelium with red secretory cells scattered among the ciliated columnar epithelial cells.

The gills varied from very good, with normal Leydig cells, secretory epithelium along the water tubes, and little amebocytic infiltration of the connective tissue or dilation of the blood vessels, to those in which there was a moderate amount of amebocytic infiltration, dilation of the blood vessels, and some disruption of the supporting connective tissue of the

TABLE 2. Histopathological Comparison of the Four Groups of Oysters.^a

Tissues	Controls	Empire Mix	Saudi Arabian	Nigerian
Reproductive tract	4	4	2	1
Hyaline degeneration	4	2	4	1
Leydig cell disruption	3	3	2	1
Digestive diverticula	3	3	2	1
Brown cells with dark granules	4	3	2	1
Periostracum growth	4	3	2	1
Number and types of parasites	3	2	3	1

^aRating: Good = 4; Poor = 1.

interfilamentary and interlamellary junctions.

There was a light to moderate number of brown cells throughout the connective tissue, particularly along the intestine in some animals. A few animals had an accumulation of brown granules along the mantle edge. The bright pink-staining area in the folds of the mantle which secretes the periostracum was present and normal in all animals. The body walls and mantle were normal, as was the basement membrane, muscle, and connective tissue of the mantle and food groove.

Four of the oysters had one or two spores believed to be those of the gregarine, *Nematopsis* sp., in the connective tissue of the mantle area. Three animals had one or two small red-staining nematodes in the tubules of the digestive diverticula, which caused some tubular destruction. One animal had a few foci of large basophilic inclusions in the intestinal epithelium, which may be a mycoplasma infection such as described by

Harshbarger et al. (1977) (Fig. 4). In three of the animals a few ciliates were seen between the gill filaments, and in one oyster, they were observed in a few of the tubules of the DD.

Saudi Arabian-treated oysters. A few of the 14 oysters examined microscopically had a few spermatozoa or ova developing along the follicular or duct walls. However, there were fewer follicles developing in the connective tissue of the gonadal area than were seen in the control animals, and the germinal epithelium did not always extend the full length of the duct or follicle.

The Leydig cells in some animals were in very good condition, with pink cytoplasm and intact cell walls throughout most of the body. Others were in much poorer condition, with many of the cell walls completely disrupted (Fig. 5). The Leydig cells in the mantle, labial palps, and outer

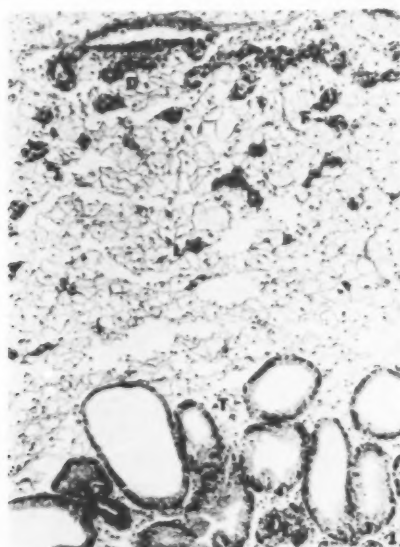


FIGURE 3. Section of the gonadal region of a female control oyster. Note germinal epithelium developing along the wall of the duct (D) and in the follicles (F). Also note the granules in the cuboidal to columnar epithelium of the tubules of the digestive diverticula (T). Letter (L) indicates the Leydig cells with all their cell walls intact. (200X)

edge of the body were the first to lose their normal architecture. This loss of normal architecture of the Leydig cells contributes to the "watery" appearance often noted in oysters after spawning, but should not have been as prevalent in these oysters since spawning season was approaching.

The digestive cells of the digestive diverticula ranged from fair to poor. Some of the tubules had a cuboidal epithelium with a few granules present, but most were low cuboidal to almost squamous, with very few

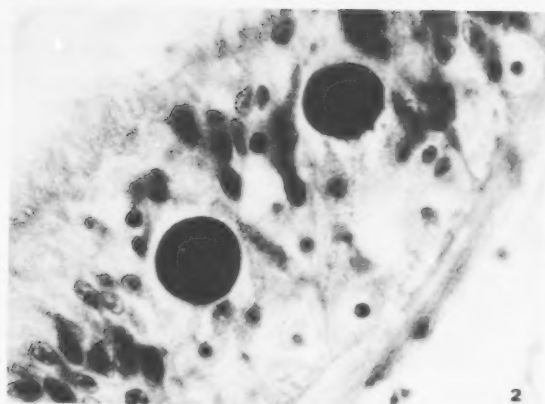


FIGURE 4. Section of the intestinal epithelium of an oyster showing two large basophilic inclusions that may be the mycoplasma infection described by Harshbarger (1977). (1260X)

granules. Some of the tubules had an increase in the number of basophilic cells. A few animals had a degeneration of the cells in the transitional area between the tubules and the ducts, with a loss of cilia on the duct epithelium. There was a general loss of red secretory granules from the duct epithelium.

The gills were generally good, similar to the controls. A few had ciliated protozoa among the gill filaments, but they did not appear to cause any cellular reaction.

The number of brown cells per oyster ranged from light to moderate-



FIGURE 5. Section of the gonadal region of a Saudi Arabian-crude oil exposed oyster. Note the lack of development of follicles in the connective tissue, the very small amount of germinal epithelium on the wall of the duct (D), and the disruption of the cell walls of the Leydig cells (L). A few medium size brown cells (B) are present. (200X)

ly heavy, with most animals being in the moderate range. Several of the animals had brown granules along the folds of the mantle and some on the mantle edge. A few animals showed no sign of periostracum development in the folds of the mantle. In others the pink-staining secretion was paler and of lesser amount than normal.

The parasites seen in this group were of the same species as seen in the controls. However, there were seven animals in this group that had the spores in the mantle connective tissue, and three of these animals had spores in the connective tissue of the body and in the muscle. As noted in

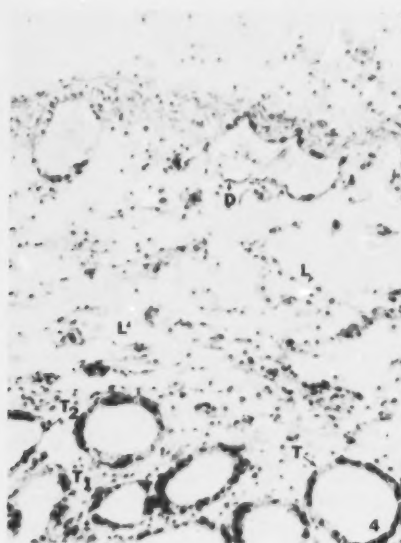


FIGURE 6. Section of the reproductive region of an oyster exposed to Nigerian crude oil. Note the lack of germinal epithelium on the gonadal ducts (D), the absence of follicles in the connective tissue area, and the disruption of the Leydig cell walls. (T) indicates a tubule of the digestive diverticula with cuboidal cells containing a few granules and some necrotic digestive cells. (T₁) indicates a tubule with extensive degeneration and necrosis of the tubular epithelium. (T₂) indicates a tubule with only the basement membrane remaining in some areas. (200X)

the controls, ciliates were seen among the gill filaments and occasionally along the body wall of three animals. Two animals had red staining nematodes in the tubules of the DD, and the large basophilic inclusions were seen in the intestinal epithelium of two of the animals.

Nigerian-treated oysters. No germinal epithelium was present in any of the 19 oysters of this group. Gonadal ducts were identifiable because of the cilia along one wall, but no follicles were observed in the connective tissue of the gonadal region (Fig. 6). Occasionally cellular debris was seen in the ducts.

The Leydig cells in most of the animals were in very poor condition, with complete loss of pink cytoplasm and rupture of cell walls throughout

most of the body. Many animals had a loss of normal epithelium from the body walls and mantle. In some of these areas there was hyaline degeneration of the collagenous connective tissue fibers, basement membrane, and muscle that lie immediately under the epithelium. With the routine hematoxylin and eosin stain and with three special stains, these areas consistently stained differently than normal tissue.

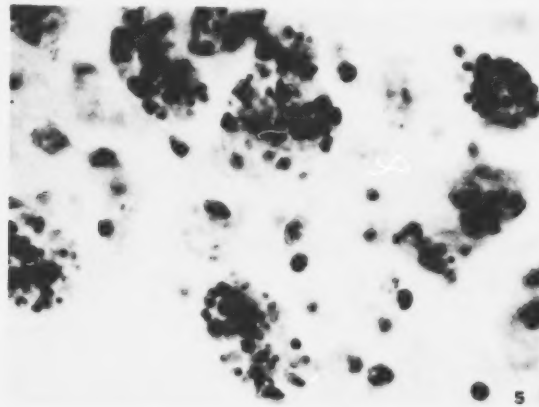


FIGURE 7. Accumulation of brown cells from an exposed oyster. Note the numerous varied colored spheres within each brown cell. (1260X)

There were many large brown cells scattered throughout the connective tissues of these oysters. The globules within the brown cells of the various animals ranged from golden brown, sometimes with a black refractile area in the center, to dark brown. Each cluster of globules making up a brown cell ranged in size from moderate to very large, containing as many as 30 to 40 spheres of varying sizes and colorations (Fig. 7). Many animals had accumulations of brown or black granules along the mantle wall and on folds of the mantle in particular. The cells responsible for the secretion of the periostracum were absent from the mantle folds of many animals.

The digestive diverticula of all of the oysters in this group were in poor condition. Some tubules had a complete loss of normal architecture; tubular epithelium ranged from squamous to almost non-existent, with only a circle of degenerating cells and a basement membrane to indicate the presence of tubules. Other tubular epithelium was entirely basophilic and without granules, or had the appearance of budding, indicating an apparent attempt to regenerate. There was necrosis and sloughing of the epithelium in some tubules and collapse of others, so that only a small circle of necrotic nuclei was left. There were very few granules present in any of the digestive cells which had not been destroyed. Much of the epithelium of the ducts appeared vacuolated. There was some loss of cilia and occasionally loss of normal cell architecture in the area between the duct and tubule.

Some of the gills of these oysters had an amebocytic infiltration, and some had dilation of the blood vessels. There was a general loss of Leydig cells and occasionally metaplasia of the columnar cells of the gill filaments. There was hyaline degeneration of the collagenous connective tissue, muscle bundles, and basement membranes underlying the food groove at the tips of the gills (Figs. 8, 9). This change was the same as that seen along the body wall and the mantle.

These animals were extensively invaded by parasites of various sorts. The number of spores of parasites observed in the mantle and throughout the connective tissue and muscle areas of the body was much greater than in the other groups of oysters studied. There were ciliated protozoal parasites along the body wall, on the gills, in the blood vessels, in the intestinal tract, and within the ducts and tubules of the DD. A few of the animals had large bright pink-staining parasites in the connective tissue of the DD. All animals had numerous foci of the basophilic inclusions in the intestinal epithelium, and some were also seen in the tubules of the DD.

Empire Mix-treated oysters. There was a great deal of variation in the condition of the 17 oysters from this pond. The animals were evenly divided between those whose gonadal tissue was functioning normally with many ova or sperm present and those in which there was little or no germinal epithelium.

The condition of the Leydig cells in these oysters was equally variable, ranging from very good, with pink cytoplasm present and cell walls intact, to a condition in which there was no pink cytoplasm and most of the cell walls were disrupted. The tubules of the DD of these animals also varied from those that had cuboidal to columnar epithelium containing many granules to those that had a squamous epithelium with no granules or a complete loss of normal architecture and amebocytic infiltration. Some of the ducts had a low columnar to cuboidal epithelium, which often appeared vacuolated.

There was a light to moderate number of brown cells per animal. Some had black or brown granules along the mantle edge. One animal had hyaline degeneration of the food groove area, and another had a similar change along the mantle edge. The gills of these animals were similar to those of the controls.

Most of the oysters had spores of parasites in the connective tissue, particularly in the mantle area, but they were not as numerous as they had been in the Nigerian-treated animals. Three of the oysters had basophilic inclusions in the intestinal epithelium, but again they were not as numerous as in the Nigerian animals. Two oysters had an extensive infiltration of larval trematodes, probably *Bucephalus* sp., in the DD, labial palps, blood vessels, and reproductive tract.

DISCUSSION

If mortality data is considered in conjunction with the histopathological observations made on oysters which survived exposure to the respective oils, it must be concluded that the chronic exposure to low

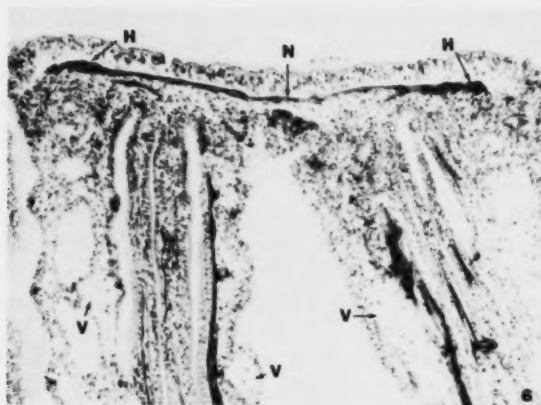


FIGURE 8. Section of the food groove area at the tip of the gills of a Nigerian crude oil exposed oyster. This slide was stained with Heidenhain's Azocarmine/Aniline Blue method for connective tissue. Note the appearance of the normal area (N), which stains a medium blue with Heidenhain's, versus the swollen dense mass of the area of hyaline degeneration, which stains a bright red (H). Also note the dilation of the blood vessels of the gill filaments (V).

levels of Saudi Arabian and Nigerian, and to a lesser extent Empire Mix, caused serious alterations in the normal physiology and histology of these animals. The exact role of oil in causing these alterations is as yet unclear.

One of the most important findings of this study was the interference with the development of the reproductive tract. That oil interferes with the reproductive cycle in marine animals had been shown by Rinkevich

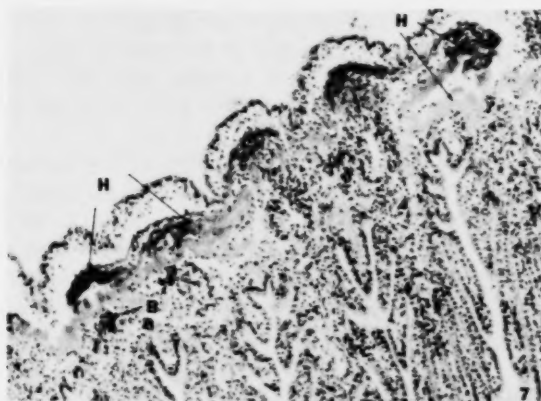


FIGURE 9. Section of the food groove area of a Nigerian exposed oyster. Note the areas that have become swollen, translucent, homogeneous, structureless masses (H). Also note the accumulation of brown cells (B) lying beneath and among the areas of hyaline degeneration. (200X)

(1977) in corals, Eisler (1975) in the gastropod drill, Kasimov and Aliev, (1973) in benthic animals of the Caspian Sea, Linden (1976) in the amphipods, and Renzoni (1975) in bivalves. These observations were based on the fecundity of the animals, but not on histopathological examination. This study shows that the lack of follicular development in the connective tissue of the gonadal region and the lack of germinal epithelium along the gonadal ducts were why the Nigerian and the Saudi Arabian crude oil-exposed animals could not reproduce. This lack of reproductive potential over a period of time would lead to the complete loss of the species from the exposed area.

The observation that the digestive diverticula of the exposed animals were similar in appearance to that seen in several species of deliberately starved bivalves from controlled experiments conducted at the Environmental Research Laboratory, Narragansett, RI, (ERL-N) coupled with no observed differences in the food available to the oysters in the respective ponds in this study (Brown, 1978), suggests that the crude oils in some way interfered with the intake and/or utilization of the food by treated oysters. This is consistent with the findings of Gonzalez (1976) and Gillfillan (1973, 1975). Poor nutrition is known to play a role in reducing fecundity, as cited by Tucker and Wyttenbach (1975).

According to Galtsoff (1964), "... resistance to infection is lowered by an unfavorable environment and an oyster weakened by adverse conditions more easily succumbs to infection." The observed increase in parasitism in the oil-treated oysters, especially those exposed to Nigerian crude, substantiates this statement by Galtsoff. Because of the poor health and lowered resistance of the animals exposed to crude oil, they are more likely to become hosts to opportunistic parasites. Also, the parasites already present have a better opportunity to increase in number. A similar increase in parasitism in juvenile flounder and *Mytilus edulis* exposed to no. 2 fuel oil at ERL-N has been observed by two of the authors (Yevich and Barszcz).

Possibly the most interesting finding of this study was the observed degeneration of the basement membranes and connective tissue and muscle fibers along the edge of the mantle and in the food groove area of the gills of many of the oil-treated oysters. These fibers were swollen, translucent, homogeneous, structureless masses which were not amyloid in nature. This condition most nearly resembled the description and appearance of hyaline degeneration as presented in pathological text books. Hyaline degeneration is believed to occur due to interference with protein metabolism. While we believe that the condition observed in oysters is a case of hyaline degeneration, we are unable at this time to explain how the oil interfered with protein metabolism. This, to our knowledge, is the first case of hyaline degeneration reported for invertebrates exposed to pollutants. Two of the authors (Yevich and Barszcz) have observed but not yet reported hyaline degeneration in the basement membrane and connective tissue/muscle area under the epithelial cells of the mantle of snails (*Nassarius obsoletus*) following laboratory exposure to 1 ppm of no. 2 fuel oil for five months. This condition has also been

observed in snails examined following the Amoco Cadiz oil spill in France in March of 1978.

This study underscores the importance of not treating all crude oils as the same in regard to their potential for damaging the aquatic environment. The chronic effects of Empire Mix crude oil were far less severe than those caused by Nigerian and Saudi Arabian oil.

In summary, data from this study indicate that chronic field exposure of oysters to low levels of Nigerian and Saudi Arabian and, to a lesser extent, Empire Mix crude oils can cause severe alterations in the histology of oysters. Data from the present study seem to indicate that the crude oils in some way interfere with food intake and/or assimilation; that they cause changes in the connective tissue in the body wall, mantle, and food groove areas; and that they cause reductions in the germinal tissues, reducing and/or eliminating the oyster's ability to reproduce.

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